ISOLATION AND CHARACTERIZATION OF TWO β_1-GLYCOPROTEINS OF HUMAN SERUM

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By immunoelectrophoretic criteria at least four different proteins with β_1-mobility can be distinguished, not including the β-lipoprotein (1). So far, only one, the iron-binding protein, has been isolated and thoroughly studied (2). Consequently there remain at least three β_1-globulins which are as yet unidentified.

Two of the unidentified β_1-components became of particular interest when initial observations strongly suggested that these two components were related to the complement complex. In the course of the ensuing investigation one of the proteins was disclosed as a serologically active compound and the other as its inactivated form. The results of experiments performed in a study of its serological activity are described in the following paper (3).

This paper describes the procedure of isolation, and some physical, chemical, and immunological characteristics of the two proteins. Isolation was accomplished chiefly by chromatography on anion exchange cellulose. Immunoelectrophoresis was employed to follow the process of purification because these components were originally detected by this technique. Both proteins were obtained in highly purified form. They were found to be glycoproteins which were immunologically related to each other but which differed conspicuously in their sedimentation coefficient.

Materials and Methods

Plasma and Serum Samples.—For the isolation of β_{1C}-globulin samples of pooled and individual fresh serum were used as starting material. The serum was obtained from healthy blood donors on the day the preparation was begun.

Starting material for the preparation of β_{1A}-globulin consisted of pooled, normal plasma which was furnished by the Blood Donor Service, University Hospital, Uppsala, where it was stored under sterile conditions at 4°C. for approximately 3 weeks prior to use. Preceding the precipitation of euglobulin, fibrinogen was removed by dialysis of the plasma against 0.1 per cent $CaCl_2$ in physiological saline at 2°C. for 24 hours, and subsequent centrifugation.

Euglobulin Preparations.—The euglobulin fraction containing β_{1C} was precipitated from 250 to 300 ml. portions of fresh serum by dialysis against 10 liters of phosphate buffer pH 5.4, ionic strength 0.02. Dialysis was limited to 20 hours and carried out at 2°C. The pre-

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cipitated protein representing approximately 2.5 per cent of the serum proteins was collected by centrifugation and washed twice with 250 ml. portions of ice cold phosphate buffer. Care was taken to achieve a fine suspension of the precipitate during the washing procedure. The washed precipitate was dissolved in 10 ml. 0.03 M pH 8 phosphate buffer (initial buffer for subsequent chromatography) containing 1 per cent NaCl. To remove the bulk of the lipids the euglobulins were subjected to centrifugation at 59310 *g* and 0–5°C. for 2 hours in a preparative Spinco ultracentrifuge. Thereafter the clear or at times slightly opalescent solution was collected through a small hole pricked into the bottom of the lusteroid centrifuge tube while the lipid layer on top was discarded.

The euglobulin fraction containing β_{1A} was prepared from 350 ml. portions of serum (obtained from stored plasma) by dialysis against phosphate buffer, pH 5.8, ionic strength 0.005, for 36 to 48 hours at 2°C. The precipitate, comprising 3 to 4 per cent of the serum proteins, was washed twice and then dissolved in 10 ml. barbital buffer, pH 8.6, ionic strength 0.1 (buffer used for subsequent electrophoresis) and centrifuged at 105400 *g* for 6 hours for removal of lipids. The resulting clear protein solution was separated from a thick lipid top layer as described above. The lipids were discarded.

Electrophoresis.—Preparative zone electrophoresis was performed using as supporting medium polyvinyl chloride resin (geon 426, Goodrich Chemical Company) (4). This method was employed to attain a partial purification of β_{1A}-globulin. Usually 10 ml. of a 5 to 6 per cent euglobulin solution was applied to a block measuring 50 x 30 x 1 cm. Electrophoresis was performed for 36 hours using barbital buffer, pH 8.6, ionic strength 0.1, and a potential gradient of 4 to 5 V/cm.

Chromatography.—Triethylaminoethyl (TEAE) cellulose anion exchanger (5) was used as adsorbent in both gradient and step-wise development procedures. Prior to each experiment the ion exchanger was treated with several volumes of 1 N NaOH, sufficient distilled water to render the pH neutral, 400 ml. 0.2 M Na_2HPO_4, 400 ml. 0.2 M NaH_2PO_4, distilled water, and 0.03 M phosphate buffer, pH 8.1, in that order. The column was packed and equilibrated overnight with the starting buffer: 0.03 M phosphate buffer, pH 8.1 (710 ml. 0.2 M Na_2HPO_4 + 31.2 ml. 0.25 M NaH_2PO_4 + distilled water to a final volume of 5000 ml.). This elaborate treatment was found necessary to ensure equilibration of the adsorbent with both phosphate ons used later during development and thus to obviate chromatographic artifacts.

During gradient development both pH and ionic strength were changed continuously. The gradient was produced using as a mixing chamber a 600 ml. beaker which contained 400 ml. starting buffer, and which was connected by a syphon with a 250 ml. Erlenmeyer flask containing 260 ml. 0.25 M NaH_2PO_4. The buffer was fed out of the mixing chamber into a 40 x 1.5 cm. column containing 65 ml. packed TEAE cellulose. The flow rate was adjusted to approximately 25 ml./hour, 3.5 ml. fractions of effluent being collected. All chromatographic experiments were carried out at 2°C. This procedure was applied to the separation of euglobulins from fresh serum to achieve a partial purification of β_{1C}-globulin. It was also used to eliminate impurities from the electrophoretically obtained β_{1A}-fraction.

Before chromatography, the protein solutions were dialysed for 3 hours against 2 x 3 liters of starting buffer, and the small amount of precipitate that usually formed during dialysis was removed by centrifugation. Of the 400 to 450 mg. of protein applied to the column, 94 to 96 per cent was recovered.

Step-wise development was employed for final purification of the β_{1C}-globulin which had been first partially purified in the gradient development procedure. From the results of the latter the exact conditions were learned at which β_{1C}-globulin can be eluted from the column. 50 to 100 mg. protein was applied to a 40 x 1 cm. column containing 20 to 30 ml. adsorbent equilibrated with 0.03 M phosphate buffer, pH 8.1. The buffers used as developer in the subsequent steps were prepared from starting buffer (A) (see above) and 0.25 M NaH_2PO_4 (B). In the first step at least 175 ml. of 0.059 M phosphate, pH 6.67 (400 ml. A + 60 ml. B) were

passed over the column to remove impurities while β_{1C} was yet fully retained. In the second step β_{1C}-globulin was eluted with approximately 250 ml. 0.079 M phosphate, pH 6.35 (300 ml. A + 85 ml. B). Two further steps were added to elute those components which remained adsorbed during development of β_{1C}-globulin. The third step was carried out with 0.13 M phosphate pH 5.8 (100 ml. A + 84 ml. B) and the fourth step with 0.195 M phosphate pH 5.1 (100 ml. A + 290 ml. B).

Immunoelectrophoresis.—Immunoelectrophoresis was performed according to Scheidegger (6) with antisera from the rabbit produced by the Behringwerke A.G., Marburg, Germany (7). Electrophoresis was carried out for 2 hours employing a potential gradient of 5.5 V/cm. 36 to 48 hours was allowed for the precipitin lines to develop before photographic records were made.

Ultracentrifugation.—β_{1C}- and β_{1A}-globulin and euglobulin preparations were analyzed ultracentrifugally using a Spinco model E machine with automatic temperature control. The proteins were dissolved in phosphate buffer, pH 7.08, ionic strength 0.1, and analyzed at 59780 R.P.M. and at 20°C. and at concentrations ranging from 2 to 10 mg./ml.

Chemical Analysis.—Quantitation of protein bound carbohydrate was performed according to methods described earlier (4).

RESULTS

Different Immunoelectrophoretic Composition of the β_1-Globulin Fraction of Fresh and Stored Sera.—

A series of forty normal sera obtained from different individuals was subjected to immunoelectrophoretic analysis. From exploratory experiments it was learned that the composition of the β_1-globulins varied characteristically with the age of the serum sample tested. Therefore, the series was arranged to include 15 fresh sera, which were analyzed within 1 day after their collection, 15 stored sera, and 10 "aged" sera, which were examined after they were kept at 1°C. for 1 to 3 weeks and 1 to 3 months, respectively. In addition some individual sera were analyzed repeatedly after varying periods during which they remained at 1°C.

The immunoelectrophoretic pattern of stored sera (1 to 3 weeks at 1°C.) invariably showed four arcs in β_1-position (Fig. 1 (*c*)). The main component has previously been identified as the iron-binding protein (1, 8). The other three represent unidentified β_1-globulins. Since they have not yet been designated they will provisionally be referred to below as β_{1A}-, β_{1B}-, and β_{1C}-globulin. β_{1A}-globulin denotes the electrophoretically fastest, β_{1C}-globulin the slowest of the three unknown components. In agar, β_{1A}- and β_{1B}-globulin migrate faster than transferrin, β_{1C}-globulin somewhat slower. It was with respect to β_{1A}- and β_{1C}-globulin that both fresh and aged sera differed distinctly from the described pattern. The patterns of all fresh sera examined lacked the "β_{1A}"-line (Fig. 1 (*a*)) and those of all aged sera (stored at 1°C. for 1 to 3 months) were devoid of the "β_{1C}"-line (Fig. 1 (*b*)). The difference between fresh, stored, and aged sera was striking since their patterns in all other details appeared to be quite similar (with the possible exception of lines ascribed to the serum lipoproteins). When the same serum was examined repeatedly in 5-day intervals, it was observed that the concentration of β_{1A}-globulin gradually increased as the amount of β_{1C}-globulin diminished.

These observations indicate that when serum is stored, β_{1C}- globulin is subject to a slowly progressive, characteristic change that results in the formation of β_{1A}-globulin as the only or the main product. The speed of this change was found to be temperature-dependent. At 1°C. the process was completed after approximately 4 weeks. At 37°C. only 48 to 72 hours were required to obtain the same effect. Heating of fresh serum at 56°C. for 20 minutes, however, did not visibly affect β_{1C}-globulin.

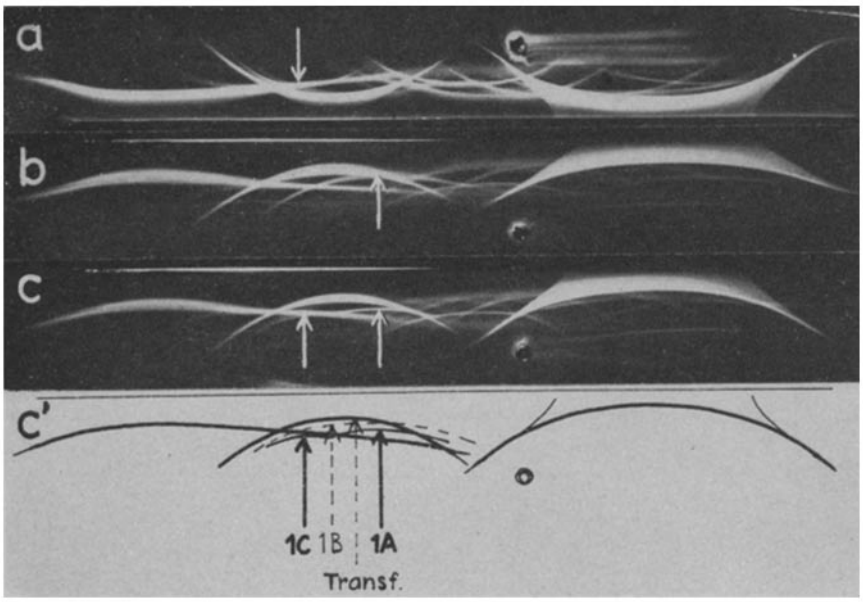

FIG. 1. Immunoelectrophoretic presentation of β_{1C}- and β_{1A}-globulin in different samples of whole serum. (*a*) fresh serum; (*b*) aged serum (stored at 1°C. for 4 weeks); (*c*) serum stored at 1°C. for 10 days; (*c'*) schematic drawing of (*c*). The arrows indicate the maximum of the arcs of β_{1C}- and β_{1A}-globulin, respectively. Patterns were developed with rabbit anti-human serum.

Isolation of β_{1A}-Globulin.—Preliminary immunoelectrophoretic experiments revealed that, while β_{1B}-globulin and transferrin remain completely in solution, β_{1A}- and β_{1C}-globulin precipitate from serum together with the euglobulins. Preparation of the euglobulin fraction from serum was therefore adopted as the first step of the isolation procedure.

The euglobulins containing β_{1A}-globulin were prepared from 350 ml. portions of serum (obtained from stored plasma) by dialysis against phosphate buffer pH 5.8, ionic strength 0.005. After the bulk of the lipids had been removed from the washed and redissolved precipitate by ultracentrifugation, the material

was separated by preparative zone electrophoresis. This resulted in a substantial purification of β_{1A}-globulin. The component was localized in the eluates from the polyvinyl block with the aid of immunoelectrophoresis. The distribution of β_{1A}-globulin was found to coincide with a major electrophoretic fraction (Fig. 2, segment 21–25), whose mobility was intermediate to that of

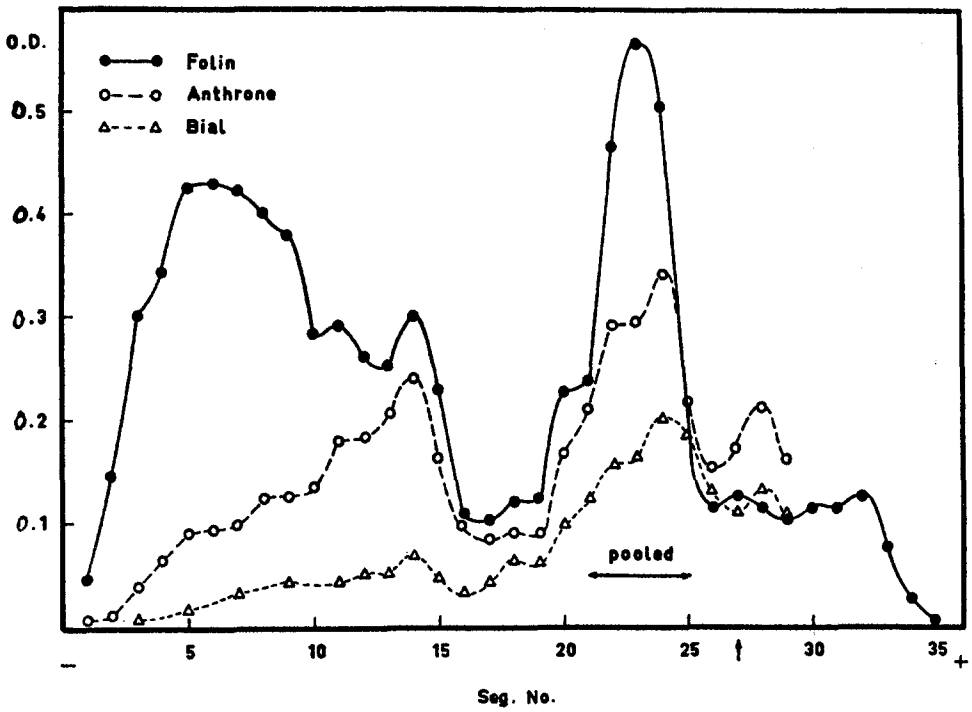

FIG. 2. Electrophoretic pattern of euglobulins containing β_{1A}-globulin separated in a polyvinyl chloride medium. By immunological analysis β_{1A}-globulin was detected in fractions 21–25, (partially purified β_{1A}-globulin).

the β- and α_2-peak of normal serum. Although it migrated as a rather narrow peak with a symmetrical distribution, this fraction was chemically heterogeneous, as was demonstrated by the distribution of protein-bound carbohydrate. The hexose- and sialic acid curves did not follow the protein curve. Tested immunologically, the electrophoretic fraction consisted of three different protein components.

Final purification of β_{1A}-globulin was achieved by means of chromatography. The electrophoretic fraction containing β_{1A}- globulin (segment 21–25 in Fig. 2) was concentrated to approximately 5 ml., dialyzed against initial chromatography buffer and applied to a column of TEAE-cellulose anion exchanger.

The chromatogram was developed using an ionic strength and pH gradient. By this procedure β_{1A}-globulin was separated from other, contaminating proteins of similar electrophoretic mobility. Fig. 3 depicts such a chromatogram, in which the major fraction represents highly purified β_{1A}-globulin which emerged from the column at pH 6.7. Effluent fractions were pooled as indicated in Fig. 3, and the protein solution was concentrated for ultracentrifugal, chemical and

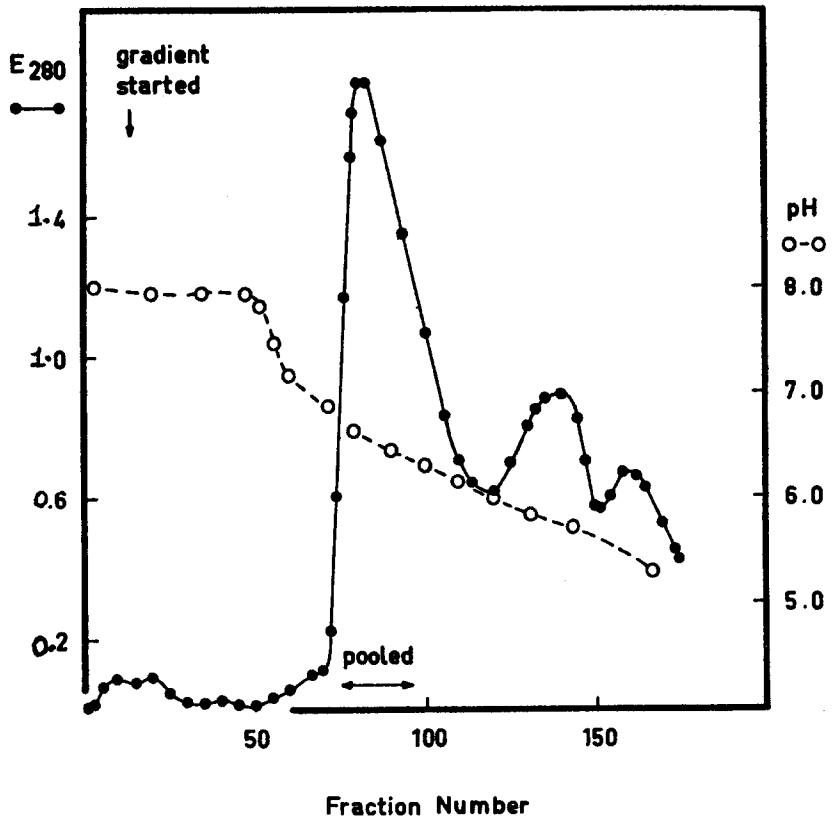

FIG. 3. Chromatographic separation on TEAE-cellulose of crude β_{1A}-globulin obtained by preparative electrophoresis (Fig. 2). Fractions pooled as indicated contained highly purified β_{1A}-globulin.

immunological studies. The yield of β_{1A}-globulin was 0.2 per cent of the total serum protein.

Isolation of β_{1C}-Globulin.—Because β_{1C}-globulin is a rather labile compound it was isolated as quickly and gently as possible. It was not possible, however, to obtain highly purified β_{1C}-globulin by a single chromatographic separation of a euglobulin preparation, using either the gradient or the step-wise development

procedure. An ultracentrifugally and immunologically homogeneous preparation was not obtained unless the material was once rechromatographed. Therefore the following procedure was elaborated. In eleven subsequent experiments it proved to be very reproducible.

The euglobulin fraction of 250 ml. portions of fresh serum was prepared as described above. The material was applied to a column of TEAE cellulose equilibrated with 0.03 M phosphate

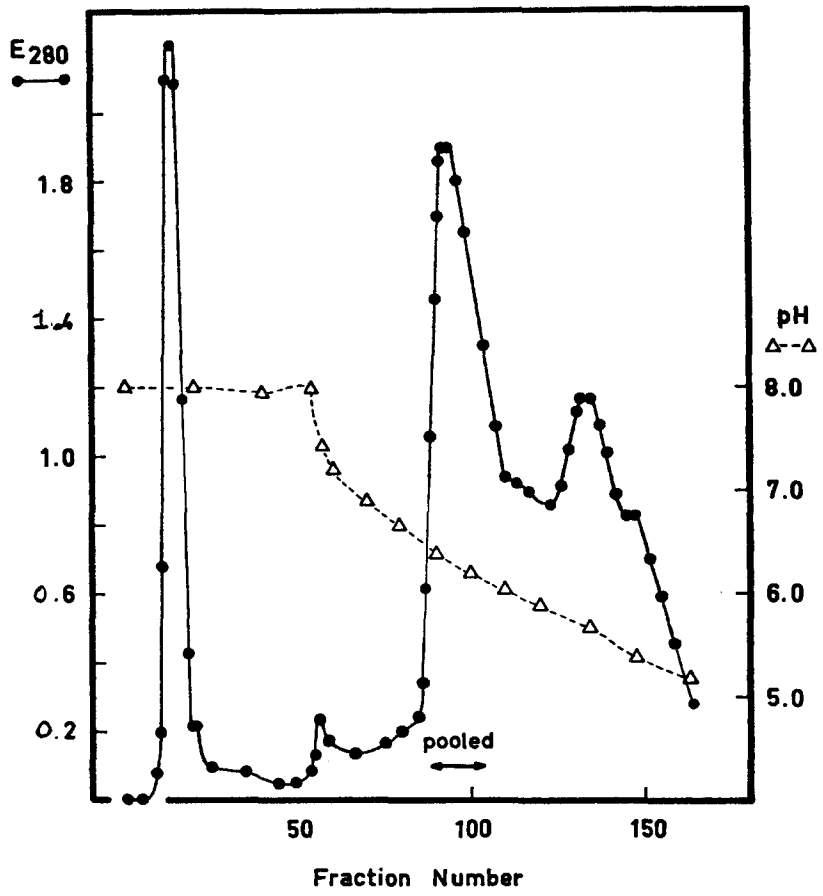

FIG. 4. Chromatogram of euglobulins containing β_{1C}-globulin. (TEAE-cellulose, gradient development). Fractions pooled as indicated contained crude β_{1C}-globulin.

buffer pH 8.1. The chromatogram was developed by the gradient development procedure. β_{1C}-globulin detected in the effluent by immunological techniques left the column approximately at pH 6.4. Effluent fractions containing β_{1C}-globulin were pooled as indicated in Fig. 4. This component represented 60 to 80 per cent of the protein in the pooled fractions.

The chromatographic fraction indicated in Fig. 4 was rechromatographed using the stepwise development procedure (Fig. 5). The latter method allowed complete separation of

β_{1C}-globulin from contaminating proteins which in the gradient chromatogram were apparently overlapping. To obtain a highly purified preparation it was found essential to allow a sufficient volume of pH 6.67 phosphate buffer to pass the column prior to elution of the desired component. In the experiment illustrated by Fig. 5 the amount of contaminating protein which can be eliminated by this step was small. But in other experiments the amount of this protein was appreciable and in such cases elution of β_{1C}-globulin was begun only when the UV absorption of the effluents in this preceding step was approaching base line values. Under these conditions β_{1C}-globulin was the sole component leaving the column when the pH was shifted to 6.35 and the molarity of phosphate to 0.079.

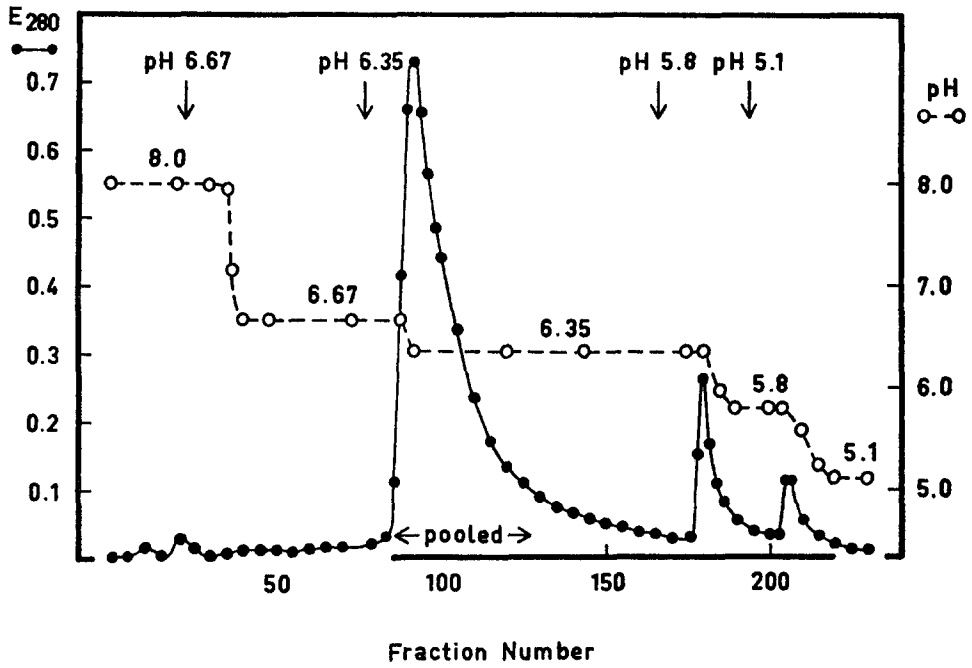

FIG. 5. Chromatogram of crude β_{1C}-globulin obtained as illustrated in Fig. 4. (TEAE-cellulose, step-wise development). Protein eluted at pH 6.35 represents highly purified β_{1C}-globulin.

β_{1C}-globulin tends to form thread-like precipitates when a solution of this protein is mechanically agitated, diluted, frozen, and thawed or concentrated by ultrafiltration. For this reason column fractions containing this protein from the first and the second chromatography experiment were not concentrated by ultrafiltration. Instead, the protein was precipitated by dialysis against phosphate buffer, pH 5.4, ionic strength 0.02, and redissolved in a few milliliters of phosphate buffer, pH 7, ionic strength 0.1. The yield of β_{1C}-globulin was 0.1 to 0.2 per cent of the total serum protein.

Immunological, Physical, and Chemical Studies on the Isolated Proteins.— Identity of the isolated products with the corresponding components in whole

serum was established in immunoelectrophoretic experiments. As shown in Fig. 6, the two isolated proteins migrate in agar with the same electrophoretic mobility and form precipitin lines of the same appearance as their analogous components in the reference sera. In another type of immunoelectrophoretic experiment one of the proteins was allowed to diffuse from one side and the antiserum from the other side towards serum previously separated by electrophoresis. The resulting straight precipitin line of the isolated protein extended parallel to the direction of migration of the serum, and was deviated towards

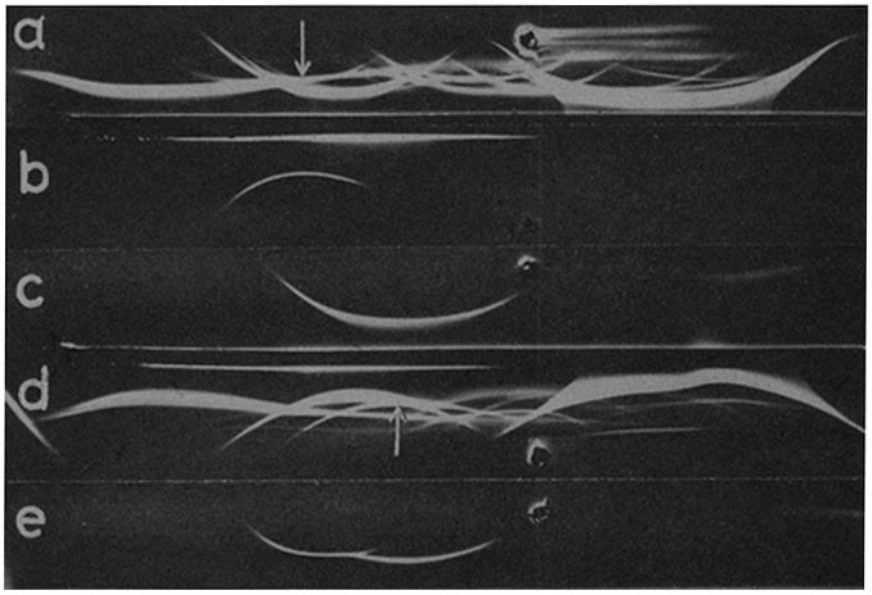

FIG. 6. Comparative immunoelectrophoretic analysis of the purified β_1-globulins and reference sera. (*a*) fresh serum, (arrow indicates the maximum of the β_{1C}-arc); (*b*) isolated β_{1C}-globulin; (*c*) isolated β_{1A}-globulin; (*d*) aged serum, (arrow indicates the maximum of the β_{1A}-arc); (*e*) combination of the isolated proteins. Developed with anti-human serum.

and completely fused with the arc of the same component in whole serum. The results indicate that, as far as can be judged by this method, β_{1C}- and β_{1A}-globulin did not undergo any change in the course of their isolation. Fig. 6 also demonstrates the degree of purity of both preparations. Only single precipitin lines were observed, even after periods of incubation up to 5 days. In view of the finding (3) that serological activity is associated with preparations of β_{1C}-globulin, two such preparations were scrutinized with respect to possibly present minor impurities. They were tested by Ouchterlony's double diffusion technique (9) against anti-normal serum and against potent specific antisera to γ-globulin, β-lipoprotein, α_2-macroglobulin, and fibrinogen. There was no

evidence of impurities detectable by these antisera and by this method. As yet no attempts have been made to produce specific antisera to the isolated proteins.

Although the question of stability has not yet been investigated thoroughly, preliminary observations indicate that β_{1C}-globulin possesses a greater stability in the isolated state than in serum. When the isolated protein was stored at 1°C. in phosphate buffer pH 7.08, ionic strength 0.1, it retained its immunoelectrophoretic appearance for at least 4 weeks. Finally, however, it assumed

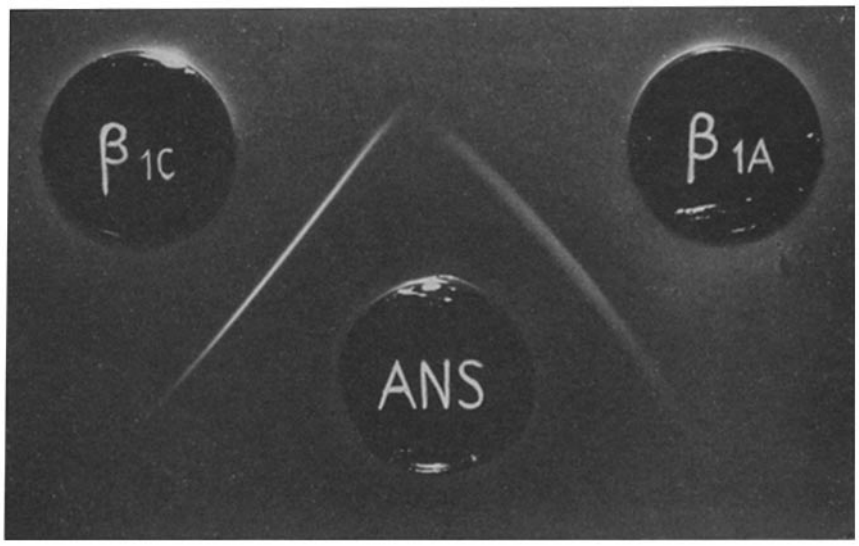

FIG. 7. Ouchterlony plate showing the reaction of partial identity between β_{1C}- and β_{1A}-globulin. (Protein concentration: 0.5 mg./ml.; ANS: anti-normal serum).

the characteristics of β_{1A}-globulin. At 37°C. conversion was completed after 6 days.

The distinction between β_{1C}- and β_{1A}-globulin so far was founded on the difference in electrophoretic mobility. The immunological examination disclosed that they also differ with respect to antigenicity, though only to a certain degree. As demonstrated in Fig. 6 (*e*) and Fig. 7, they give the reaction of partial identity. The β_{1A}-line fuses completely with the β_{1C}-line whereas the latter extends a fine but gradually increasing spur beyond the site of coalescence; *i.e.*, both are related antigens but β_{1A}-globulin is antigenically deficient as compared to β_{1C}-globulin.

A further difference was indicated by the observation (Fig. 7) that the β_{1A}-line was slightly concave toward the antibody well and the β_{1C}-line toward the antigen well. This finding suggests a difference in rate of diffusion and hence in molecular weight (10).

The assumption was corroborated by the results of ultracentrifugal studies. The sedimentation coefficient for β_{1C}-globulin was found to be $S^0_{20,w}$ = 9.5 S and for β_{1A}-globulin $S^0_{20,w}$ = 6.9 S. As demonstrated in Fig. 9 the s rate of the former was less concentration-dependent than that of the latter. Fig. 8 depicts the ultracentrifugal patterns of β_{1C}- and β_{1A}-globulin. In both instances

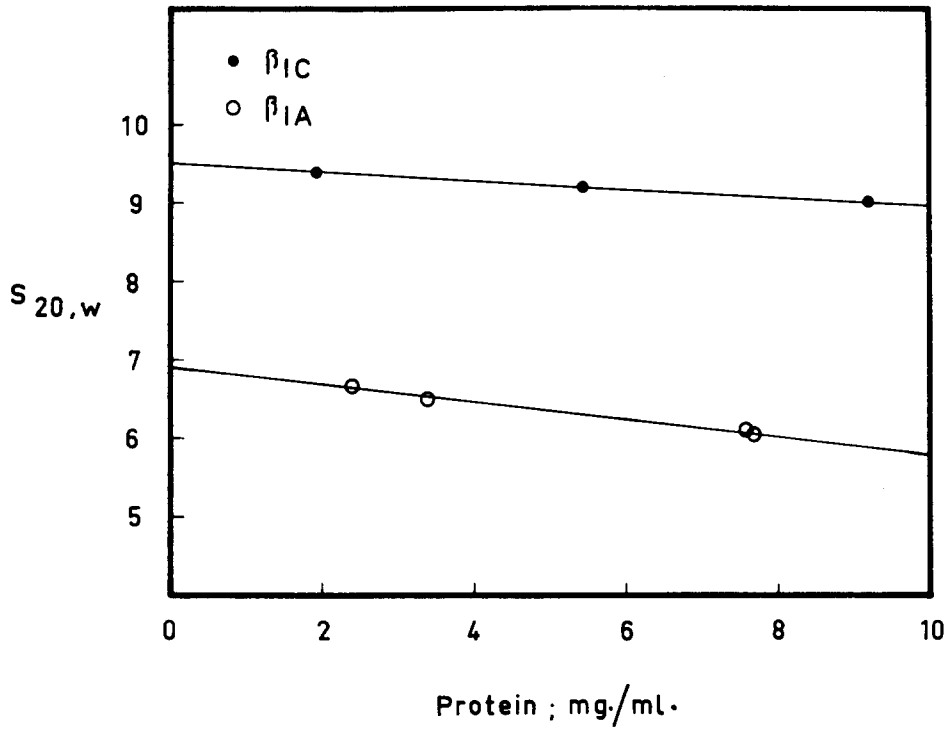

FIG. 8. The concentration dependence of the sedimentation constant for β_{1C}- and β_{1A}-globulin. By least square analysis, $y = -0.05\ x + 9.50$ for β_{1C}, and $y = -0.11\ x + 6.90$ for β_{1A}.

only a single component was observed, and this remained of symmetrical appearance throughout sedimentation.

To exclude the possibility that the 9.5 S component was derived from material of higher or lower s rate by either dissociation or aggregation during chromatography, the euglobulin preparation used as starting material for the isolation of β_{1C}-globulin was examined. It was found to contain a distinct component with an s rate of 9 to 10 S, which accounted for approximately 15 to 20 per cent of the total protein. On the other hand, euglobulin preparations from stored serum, which served as starting material for the isolation of the 7 S-β_1-globulin, lacked a 9.5 S component.

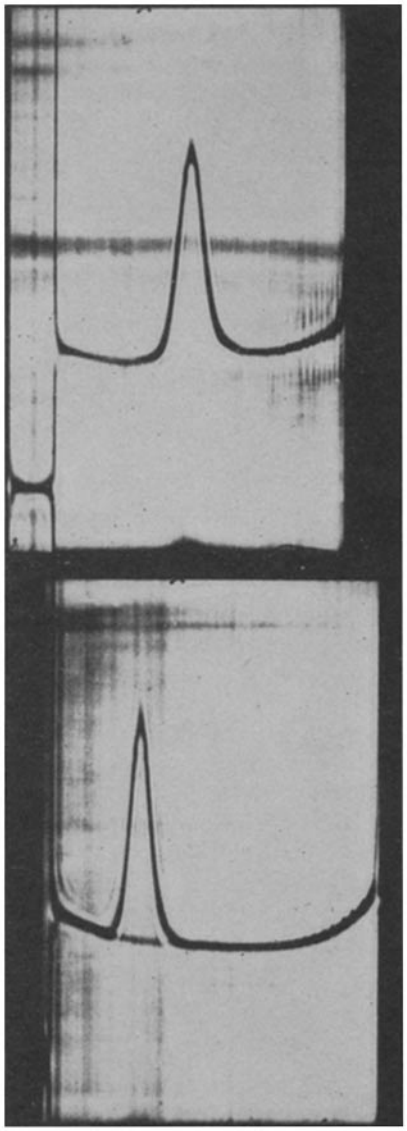

FIG. 9. Ultracentrifugal patterns of β_{1C}-globulin (upper) and β_{1A}-globulin (lower). Direction of sedimentation to the right. Proteins were dissolved in phosphate buffer, $\mu = 0.1$ and examined at comparable concentrations and at 59780 R.P.M. Photographs taken after 48 minutes.

To investigate whether β_{1C}-globulin could be dissociated with sulfhydryl compounds to a 7 S protein resembling β_{1A}-globulin, a purified preparation of β_{1C}-globulin was treated with 0.1 M mercaptoethanol at pH 7.08 and room temperature for 24 hours. After this treatment 80 per cent of the protein retained its characteristic s rate of 9.5 S, while 20 per cent sedimented more rapidly; no 7 S-component was observed.

Two preparations of the same degree of immunological and ultracentrifugal purity demonstrated above were analyzed for various carbohydrates and for nitrogen. The data are listed in Table I. All carbohydrate analyses were carried out in duplicate. The figures referring to nitrogen content represent single determinations. Considering the experimental error of the methods (4), no significant difference between the two proteins could be detected. Neither of

TABLE I

Carbohydrate Content of Two β_1-Globulins

Protein	N	Hexose	Fucose	Hexosamine	Sialic acid	Total carbohydrate	$\frac{\text{Hexosamine}}{\text{Hexose}}$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
β_{1C} (9.5 S)	15.5	1.80	0.16	0.48	0.45	2.73	0.26
β_{1A} (7 S)	15.9	1.70	0.15	0.55	0.42	2.67	0.33

them contained lipids as judged by the absence of cholesterol. Thus β_{1C}- and β_{1A}-globulin represent glycoproteins with a relatively low and very similar carbohydrate content.

DISCUSSION

In 1947 Oncley and associates (11) described two lipid-free β_1-euglobulins of human serum with sedimentation rates of 7 S and 20 S, respectively. These authors did not mention the occurrence of a 9.5 S β_1-euglobulin. They did, however, describe several components with s rates of 9 to 10 S; one of these was found to belong to the α_2-group, another to the γ-globulin fraction, and a third to fibrinogen. The 10 S γ-globulin was assumed to be produced by aggregation of 7 S molecules.

The β_{1C}-globulin described in this paper as a 9.5 S glycoprotein with β_1-mobility and with solubility properties of a euglobulin does not seem to be identical with any of the above proteins. It differs from the β_1-euglobulins characterized by Oncley and associates with respect to its s rate and is distinguished from the other known 9 to 10 S components of plasma by its antigenic and electrophoretic properties. Thus, β_{1C}-globulin appears to be distinct from previously described proteins with which it shares one or another physical property. Its tendency to change to a compound with different physical proper-

ties on aging, and its readiness to denature when manipulated, may have prevented its earlier recognition.

The concentration of β_{1C}-globulin in serum could not be accurately measured. Available data suggest that it accounts for 15 to 20 per cent of the euglobulins which, when prepared as outlined above, represent approximately 2.5 per cent of the serum proteins. Hence, β_{1C}-globulin is estimated to constitute 0.5 per cent of the total serum proteins. That the yield under the employed experimental conditions is less than half that value must be attributed to the following factors: omission of column fractions containing only small amounts of β_{1C}-globulin, incomplete reprecipitation of the protein from pooled column effluents, and denaturation of small portions of the protein during the entire procedure.

The immunoelectrophoretic and ultracentrifugal observations indicated that β_{1C}-globulin as present in serum and in its isolated state was converted to a protein of lower *s* rate, greater electrophoretic mobility and somewhat different immunological properties. This was designated β_{1A}-globulin. Whether the latter is produced only *in vitro*, or also occurs *in vivo*, is not known. It appeared to be homogeneous and showed no tendency to spontaneous degradation. The difference in *s* rate (9.5, 7 S) suggests either a dimer-monomer relationship between the two components or a change in molecular shape. Immunological experiments lend support to the first possibility. The characteristic curvature of the precipitin lines observed in the Ouchterlony test suggests that β_{1C}-globulin is of higher molecular weight than β_{1A}-globulin. Furthermore, when equal amounts of both proteins were tested against the same antiserum the precipitin line of β_{1A}-globulin was located closer to the antibody well, which may indicate that this protein was present in a higher molar concentration.

The conversion of β_{1C}- to β_{1A}-globulin, whatever its underlying mechanism, is of immunological importance because it leads to complete loss of the serological activity associated with intact β_{1C}-globulin (3).

SUMMARY

Two immunoelectrophoretically defined, heretofore unidentified β_1-globulins of human serum, provisionally designated β_{1C}- and β_{1A}-globulin, were isolated by means of preparative electrophoresis and chromatography on anion exchange cellulose.

The sedimentation coefficient $S^0_{20,w}$ of β_{1C}-globulin was shown to be 9.5 S, and that of β_{1A}-globulin, 6.9 S. Both proteins were found to contain similar amounts of carbohydrate, to be devoid of lipids, and to possess the solubility characteristics of euglobulins. In the Ouchterlony double diffusion test they gave the reaction of partial identity, which revealed β_{1A}-globulin to be antigenically deficient as compared to β_{1C}-globulin.

β_{1A}-globulin could not be detected in fresh sera and β_{1C}-globulin was absent from aged sera. Highly purified β_{1C}-globulin stored at 1°C. was converted to

β_{1A}-globulin within 4 to 6 weeks, and at 37°C. was converted within 6 days. The likelihood of a dimer-monomer relationship between these two proteins was discussed.

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BIBLIOGRAPHY

1. Grabar, P., The use of immunochemical methods in studies on proteins, *Advances Protein Chem.*, 1958, **13,** 1.
2. Koechlin, Bernard A., Preparation and properties of serum and plasma proteins XXVIII. The β_1-metal-combining protein of human plasma, *J. Am. Chem. Soc.*, 1952, **74,** 2649.
3. Müller-Eberhard, H. J., and Nilsson, U. Relation of a β_1-glycoprotein of human serum to the complement system, *J. Exp. Med.*, 1960, **111,** 217.
4. Müller-Eberhard, H. J., and Kunkel, H. G., The carbohydrate of γ-globulin and myeloma proteins, *J. Exp. Med.*, 1956, **104,** 253.
5. Porath, J., Some cellulose ion exchangers of low substitution and their chromatographic application, *Ark. kemi*, 1957, **11,** 97.
6. Scheidegger, J. J., Une micro-méthode de l'immuno-électrophorèse, *Internat. Arch. Allergy*, 1955, **7,** 103.
7. Schultze, H. E., and Schwick, G., Immunchemischer Nachweis von Proteinveränderungen unter besonderer Berücksichtigung fermentativer Einwirkungen auf Glyko- u. Lipoproteine. Immunoelektrophoretische Studien, *Behringwerk-Mitteil.*, 1957, **33,** 11.
8. Schultze, H. E., Heide, K., and Müller, H., Über Transferrin/Siderophilin (β_1-metallbindendes Globulin), *Behringwerk-Mitteil.*, 1957, **32,** 25.
9. Ouchterlony, Ö., Diffusion-in-gel methods for immunological analysis, *Progr. Allergy*, 1958, **5,** 1.
10. Korngold, L., and van Leeuwen, G., The effect of the antigen's molecular weight on the curvature of the precipitin line in the Ouchterlony technique, *J. Immunol.*, 1957, **78,** 172.
11. Oncley, J. L., Scatchard, G., and Brown, A., Physical-Chemical characteristics of certain of the proteins of normal human plasma, *J. Physic. and Colloid Chem.*, 1947, **51,** 184.